COMBINED PURIFICATION AND CONCENTRATION BY DETERMINISTIC LATERAL DISPLACEMENT WITH RECIRCULATION OF PRODUCT

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of US. Provisional Patent Application No. 62/670,839, filed on May 13, 2018.

FIELD OF THE INVENTION

[0002] The present invention is directed to methods of simultaneously purifying and concentrating cells or other particles by performing deterministic lateral displacement on microfluidic devices while recirculating product.

BACKGROUND OF THE INVENTION

[0003] Deterministic lateral displacement (DLD) typically uses two co-flowing liquids, one containing particles (the sample) and one that is a wash/collection fluid (the "running" or "wash" fluid). During DLD, particles above the critical diameter of the array are deflected into the wash fluid whereas particles below the critical diameter follow the flow direction of the sample stream. The concentration of particles in the final product (collected) stream is dependent on the input concentration of particles in the sample stream, the ratio of sample to wash fluid, and the percentage of the total volume that is collected as product. Obtaining high concentration factors using most current DLD procedures is possible but can increase the risk of contamination (particles lower than the critical diameter being collected in the product stream) or can require very long DLD arrays with extra redundancy to ensure efficient bumping into a narrow collection width.

[0004] It is also difficult to adjust the concentration of the product produced by DLD to achieve consistency on a run-by-run basis or to standardize the results obtained across multiple donor samples with different input cell counts. Samples can always be diluted, but concentration is far more challenging. Samples with different input concentrations cannot be standardized to a single output concentration without doing an up-front dilution that would be different for each sample.

[0005] Another problem with controlling product concentrations using current procedures is that relatively large volumes of wash fluid are usually required. Limiting these volumes should result in higher product concentrations, but there must always be enough wash fluid to process the entire sample volume.

SUMMARY OF THE INVENTION

[0006] General Description

[0007] The present invention is based on the concept that it is possible to control the final concentration of DLD products by recirculating product streams back onto microfluidic devices and applying them in the place of wash fluid. Initially both sample and wash fluid (also referred to herein more specifically as a wash buffer or reagent) are fed onto a device. Then, at a chosen point during the run, the inlet carrying wash fluid is sealed off, and product from an outlet of the device is fed through the inlet in the wash fluid's place. This can be accomplished using standard valves, with the recycled product stream being propelled using, for example, pressurized containers, peristaltic pumps, or syringe pumps.

[0008] During the time that collected product (P1) is being recycled onto the device, there are two processes occurring. First, sample flowing adjacent to the recycled product is being fractionated based on size such that large particles are deflected into the "wash stream" (now P1) and directed to the product outlet. Second, the number of particles in P1 is being increased thereby increasing product concentration. One way that this process may be characterized is in terms of a "concentration ratio," which is a ratio of the percentage of wash fluid in vs. percentage of product collected. A typical set of ratios might be: 60% sample and 40% wash fluid at the inlets, and 20% product and 80% waste at the outlet. Under these circumstances, if P1 is recycled as wash fluid, the particles in the wash stream will be concentrated by 2× (40%/20%) because they are still being deflected in the array and into the product collection stream. Therefore, the collected product (P2) contains both the P1 cells concentrated at 2×, in addition to the cells from the sample processed during that time period.

[0009] One advantage of this method is that the final concentration of the output product is dependent on the number of recirculations and the initial wash volume used. It is therefore easily adjustable. For example, with a donor sample that is very dilute, the operator may choose to run only a small volume of the sample against wash fluid, and then recirculate the product several times in order to process the remaining sample. As a result, a higher concentration factor may be obtained. This allows the operator (or instrumentation) to deliver any output concentration regardless of input concentration. It also means that instrumentation can be optimized to deliver a fixed product concentration needed for downstream assay steps independent of the input donor concentration. The user would simply input the initial donor concentration, and select a desired output concentration. Using this information, the instruments would adjust the number of recirculations to achieve the selected value.

[0010] Another advantage of this method is that concentration is achieved without requiring a separate concentrator device (either in-line, or run as a second pass). The concentration occurs on the same DLD used for the separation of the cells and the time needed to process the donor sample remains the same as it would be if the sample were run solely against wash buffer.

[0011] The volume of wash fluid required is also significantly less. Instead of requiring enough wash fluid to process the entire sample, the wash volume will be reduced by an amount corresponding to the volume of recycled product. For example, if recycling was initiated after about 50% of sample had been processed, the volume of wash fluid would be reduced by about half.

Embodiments

[0012] In its first aspect, the invention is directed to a method of separating target cells or target particles of a predetermined size from a sample containing cells or particles of less than the predetermined size. The method comprises applying the sample and a wash fluid to a microfluidic device at separate inlets. The wash fluid may be water or an aqueous buffer, and may optionally comprise reagents that chemically react with sample components or antibodies, carriers or activators that interact specifically with target